NCERQA STAR GRANT ABSTRACT

EPA Grant Number: R827441
Title: Fetal Metabolism of Aflatoxin B_1 and Susceptibility to Childhood Cancer
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Project Period: 7/1/99-6/30/02
Project Amount: $523,123
Research Category: Children's Vulnerability to Toxics

Objectives/Hypothesis:

Cancer is the second leading cause of death for children under fourteen years of age in the United States. The initial peak of cancer incidence occurs during the first five years of life, and available evidence indicates that a primary risk factor for childhood cancer involves transplacental exposure to either mutagenic or pro-mutagenic agents. The rapid changes that occur during fetal development may result in critical windows of susceptibility to toxic injury. A primary determinant of this susceptibility is the balance among *in utero* conversion of procarcinogens to DNA-reactive metabolites (e.g. by cytochrome P450s, lipoxygenases) and the detoxification of reactive intermediates (e.g. by glutathione *S*-transferases and other phase II enzymes). The long term objective of this proposal is to characterize the metabolism of the transplacental dietary carcinogen aflatoxin B_1 (AFB_1) in fetal liver and maternal placenta in order to understand the genetic and developmental risk factors for AFB_1 and other dietary procarcinogens. Our hypothesis is that a primary risk factor the childhood cancer human fetus is at increased risk to transplacental AFB_1-induced DNA injury due to efficient fetal activation of AFB_1 to the mutagenic metabolite AFB_1-8,9-*exo*-epoxide (AFBO), and due to inefficient AFBO detoxification. We further hypothesize that there are important interindividual and developmental differences in the biotransformation of dietary carcinogens that result in critical windows and differing susceptibility to childhood cancer.

Approach: The specific aims of this grant will be addressed by using biochemical, immunohistochemical, and molecular techniques in isolated human fetal liver tissues and culture of adult and fetal precision liver slices. We will conduct detailed *in vitro* biochemical studies to establish the kinetics of AFB_1 activation and to identify the high affinity AFB_1 bioactivation enzyme and AFBO detoxification enzymes in maternal placenta and fetal liver. Cellular targets of AFBO will be analyzed in fetal liver hepatocytes and hematopoietic precursor cells. Quantitative Western blotting will be used to determine ontogenic expression of the key AFB_1 bioactivation and AFBO detoxification enzymes among fetal donors. Studies using cultured precision fetal liver slices will determine the sensitivity of fetal tissues to AFBO-DNA binding *in situ*, and the functional relationships among fetal AFB_1 biotransformation, AFBO-DNA binding, AFBO-DNA adduct repair (by unscheduled DNA synthesis), and *p*53 tumor suppressor gene mutation frequency (by restriction fragment length polymorphism polymerase chain reaction (RFLP/PCR) analysis). The metabolism of other pertinent dietary procarcinogens may also be investigated in our model system.

Expected Results: At the completion of this project, we will understand the mechanisms and risk factors associated with a potent rodent transplacental carcinogen and probable human transplacental carcinogen positively associated with high rates of certain childhood cancers. In addition, we will have identified particularly sensitive age groups and windows of developmental susceptibility to transplacental carcinogen exposure. Once risk factors and critical susceptibility windows are identified, then appropriate risk avoidance or minimization strategies can be employed. Finally, this project generates and integrates mechanistic data based upon human tissues and cultured precision human liver slices, and thus minimizes uncertainty associated with animal data in the human risk assessment of transplacental carcinogen exposure and childhood disease.

Supplemental Keywords: (do not duplicate terms used in text) aflatoxin, heterocyclic amines, transplacental carcinogenesis, sensitive populations, *p*53, human liver slices